with no evidence of disease. Flow cytometry shows no aberrant populations. Cytogenetic testing is pending at this report. HTLV 1 viral load studies show consistent decrease in viral load. The patient is enjoying an active life with no significant post transplant adverse effects.

GVH/GVL

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HOST-REACTIVE MEMORY T LYMPHOCYTES ALONE DO NOT INDUCE MORE SEVERE GRAFT-VERSUS-HOST DISEASE

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Memory T lymphocytes are thought to mediate more rapid and profound memory immune response. In this study, we tested whether it is true in graft-versus-host disease (GVHD). C57BL/6 mice ($H2^b$) were injected with irradiated BALB/c ($H2^d$) spleen cells intraperitoneally at least 8 weeks before they were used as immunized donors. Splenic T cells (>90% pure) were harvested from these immunized animals and separated into $CD62L^+$ (>90% pure) and CD62L- (>99% pure) populations by magnetic beads. $CD62L^+$ T cells include both naive and memory T cells, while $CD62L^-$ T cells contain memory T cells but no naive T cells. We next compared the alloreactivity and the ability to induce GVHD (C57BL/6 to BALB/c) of $CD62L^-$ T cells with those of unseparated and $CD62L^+$ T cells. Despite $CD62L^-$ (memory) T cells contain similar numbers of interferon-γ-secreting cells upon response to host antigens as detected by enzyme-linked immunospot assay, $CD62L^-$ T cells had dramatically decreased ability to induce GVHD in BALB/c mice compared to unseparated and $CD62L^+$ T cell controls (Table). However, $CD62L^-$ T cell recipients did develop GVHD as documented by weight loss (Table) and histologic changes. Similar results were observed when the mice immunized with BCL1 cells (a BALB/c-origin B cell lymphoma/leukemia cell line) were used as donors. We conclude that host-reactive memory T cells alone have decreased ability to induce GVHD compared with a mixture of naive and memory T cells. Memory T cells obtained from tumor cell-immunized donors may be a unique source of T cells for tumor immunotherapy because they do not induce severe GVHD.

Groups	n	% Survival	% Weight loss
		day +100	day +100
TCD BM only	12	100%	9%
Unseparated T cells	25	4%	-
$CD62L^+$ T cells cells	4	0%	-
$CD62L^-$ T cells	9	89%	21%

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A CRITICAL ROLE FOR $CD4^+CD25^+$ SUPPRESSOR CELLS IN CONTROLLING GVHD AFTER DLI IN MURINE BONE MARROW CHIMERAS

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A murine model of allogeneic bone marrow (BM) transplantation (C57BL/6[B6]-into-AKR/J) was used to further define the role of $CD4^+CD25^+$ T cells in controling GVHD after DLI and their potential therapeutic application. B6-into-AKR BM chimeras were depleted of $CD25^+$ cells *in vivo* with anti-CD25 mAb or transplanted with BM from $CD25^{KO}$ donors so that the BM recipients would be devoid of donor-derived $CD25^+$ cells. Depletion of $CD25^+$ cells *in vivo* (>70% depletion) significantly increased the severity of DLI-induced GVHD as indicated by body weight loss and increased mortality as compared to nondepleted control mice. GVHD was even more intense in mice transplanted with $CD25^{KO}$ BM. Enhanced GVHD after CD25 depletion was seen after DLI of either 30 million or 60 million donor splenocytes. To test the ability of $CD25^+$ cells to persist and expand *in vivo*, nylon-wool enriched, immunomagnetically selected fresh $CD25^+$ cells were adoptively transferred into syngeneic nude mice. These cells progressively expanded (CD4 and CD8 subsets) over 5 months in syngeneic nude mice. Approximately 30% of the cells at 2-wk and less than 10% at 20-wk were still $CD25^+$. Various conditions for activation and expansion of $CD25^+$ suppressor cells were examined. Freshly-isolated B6 $CD25^+$ cells were cultured with rIL-2 in the presence of (1) immoblized anti-CD3 mAb plus soluble anti-CD28 mAb, (2) magnetic beads coated with anti-CD3 and anti-CD28 mAbs, and (3) CD32/4-1BB ligand double-transfected K562 tumor cells (K562DT) "loaded" with anti-CD3 and anti-CD28 mAbs. The K562DT cells induced superior short-term expansion of $CD4^+CD25^+$cells (>10 fold after 7-10 days of culture). Immoblized anti-CD3 plus soluble anti-CD28 preferentially expanded the CD8 subset from <3% in fresh $CD25^+$cells to >75% of the total cells after 1 week of culture. *Ex vivo* expanded $CD25^+$ cells were more suppressive than fresh cells added to primary mixed lymphocyte cultures in which 50% of stimulation inhibition was achieved at a 1:8 $CD25^+/CD25^-$ ratio for *ex vivo* expanded $CD25^+$ as compared to a 1:1 ratio required for fresh cells. Studies looking at the ability of *ex vivo* expanded $CD25^+$ cells to persist/expand *in vivo*, and the GVHD-protective role of freshly-isolated versus *ex vivo* expanded $CD25^+$ cells coadministered with DLI, are in progress.

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TCR TRANSGENIC HEMATOPOETIC STEM CELLS ENGRAFT INTO NON-TRANSGENIC RECIPIENTS AND EVOLVE ANTIGEN-SPECIFIC T-CELLS

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We hypothesized that genetically modified hematopoetic stem cells (HSC) transduced with a specific tumor antigen specific TCR may, when given as a fraction of a donor hematopoetic stem cell graft, lead to engraftment of tumor antigen specific T-cells and facilitate directed Graft vs Malignancy (GVL). We are developing a model system for exploring the feasibility of this approach. An important component of evaluating such a strategy is determining the ability for donor TCR-transgenic HSC to engraft into non-transgenic recipients, and to determine the kinetics of antigen specific T-cell engraftment. P14 mice are transgenic for the class-I restricted TCR specific to LCMV-Glycoprotein-33. Bone marrow was obtained from P14 CD45.2 TCR-Transgenic mice and transplanted into lethally irradiated non-transgenic, congenic CD45.1 recipients. Of the 500, 000 marrow cells in the graft, 0%, 10%, 25%, 50% or 100% was from a P14 donor, the remainder from CD45.1 congenic marrow. The number of LCMV-GP33 specific T-cells in the peripheral blood was measured weekly with MHC-H-2 D(b)GP33 tetramer. Control P14 mice contained an average of 25% CD8+ cells in the peripheral blood, of which 82% were GP33 tetramer positive. We found that TCR-transgenic P14 bone marrow would engraft, and result in measurable donor derived LCMV-GP specific CD8+ T-cells. The fraction of tetramer positive T-cells in peripheral blood increased in correlation to the percentage of P14 bone marrow transplanted. Four weeks post-transplant CD45.2, tetramer+ T-cells made up a mean 3.5% of peripheral blood in mice which had received 100% P14 donor marrow, 1.3% from 50% P14 donor, and .63% from a 25% P14 marrow donor. By week five, tetramer + cells had increased to 9.4% of blood cells in mice receiving 100% P14 donor marrow, 4.7% from 50% P14 marrow and .74% from 25% donor. Engraftment of tetramer+ T-cells increased in weeks 5 and 6 and then remained stable. 7 weeks post transplant in mice receiving 50% P14 marrow, 8.8% of peripheral cells were CD8+, of which 43.7% were tetramer+. 25% P14 transgenic donor recipients evolved 5.5% CD8+ T-cells of which 7.8% were tetramer positive. These results suggest that bone marrow containing TCR-